PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

First Named Inventor: Charli KRUSE

Serial No: 10/820,430 Group Art Unit: 1632

Filed: April 8, 2004 Examiner: Joanne Hama

Att. Docket No.: B1180/20026 Confirmation No.: 7174

For: ISOLATED ADULT PLURIPOTENT STEM CELLS AND METHODS FOR ISOLATING AND CULTIVATING THEREOF

FOURTH DECLARATION OF CHARLI KRUSE UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Charli Kruse, Ph.D., a citizen of Germany, hereby declare and state:

1. The resume attached as Exhibit A to my April 26, 2007 Rule 132 Declaration accurately reflects my professional credentials.

2. I am the sole inventor named in the above-identified application.

3. My research is funded in part by Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V., the assignee of the above-identified application.

4. I understand from attorneys for the assignee that claims 3, and 5-14 remain rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement for isolated pluripotent adult stem (IPAS) cells from any species of vertebrate obtained from any exocrine gland tissue, wherein said IPAS cells differentiate into any cell type, allegedly because the specification does not teach that the instant cells express cell surface markers associated with pluripotent cells, and does not teach that the instant cells exhibit a normal karyotype.

5. The specification already provides evidence of enablement with respect to two

divergent species of animal – rats and humans. I now provide evidence that the claimed invention is also enabled for a third species of animal, namely goats.

6. I and/or technicians under my direct supervision obtained IPAS cells from African Boer Goats as described below.

7. Pancreatic tissue of African Boer Goats was prepared and treated as described in the specification of U.S. App. Serial No. 10/820,430 in order to isolate pluripotent adult stem cells therefrom. After cultivating the stem cells in cell culture for 3 passages, the resulting stem cells were seeded and the differentiated cells derived therefrom were stained with antibodies against specific cell markers.

8. The differentiated cells stained positive for several cell markers having specificity for different cells of all 3 germ layers. The differentiated cells stained positive for the ectodermal cell markers GFAP and neurofilaments (see Figure 1A and 1B). The differentiated cells stained positive for the mesodermal markers collagen-II and α-smooth muscle actin (see Figure 2A and 2B). The differentiated cells stained positive for the endodermal marker cytokeranin 18 and amylase (see Figure 3A and 3B).

9. With respect to the confirmation of a normal karyotype, we enclose the results obtained by an independent cytogenetic laboratory in Kaiserslautern, Germany (attached as Appendix A). The findings of the independent cytogenetic laboratory are set forth in the summarizing opinion (see section labeled "Beurteilung", Appendix A) with respect to the specimen (translated form the German):

> Numerically and structural inconspicuous female karyotype, the satellite extension at one chromosome 22 is a normal variation without pathologic relevance.

10. Accordingly, a person reasonably skilled in the art would have been enabled by the original disclosure to isolate the IPAS cells of the claimed invention from a variety of mammalian cells, wherein the cells express cell surface markers associated with pluripotent cells, and further wherein the IPAS cells exhibit a normal karyotype, without undue experimentation.

11. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: 18.06.09

Charli Kruse, Ph.D.

Figure 1. Ectodermal Marker (GFAP, Neurofilaments, Cytokeratin)

Figure 1A. Stained with antibodies against GFAP and with DAPI

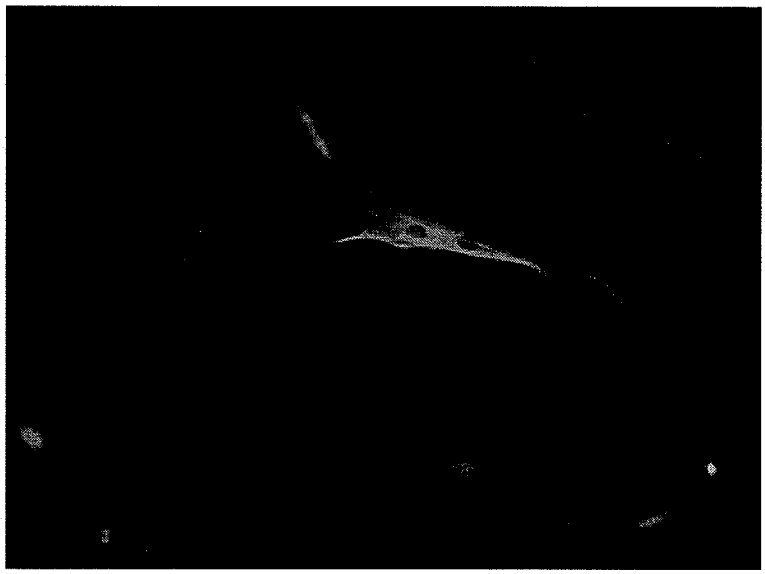


Figure 1B. Stained with antibodies against neurofilaments and with DAPI

Figure 2. Mesodermal Marker (Collagen-II, SMA)

Figure 2A. Stained with antibodies against Collagen-II and with DAPI

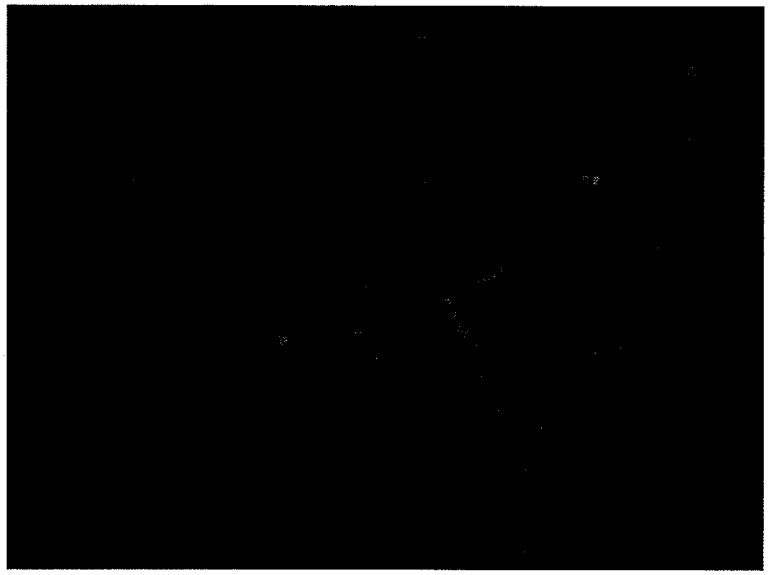


Figure 2B. Stained with antibodies against α-smooth-muscle-actin and with DAPI

Figure 3. Entodermal Marker (Cytokeratin 18, Amylase)

Figure 3A. Stained with antibodies against Cytokeratin 18 and with DAPI

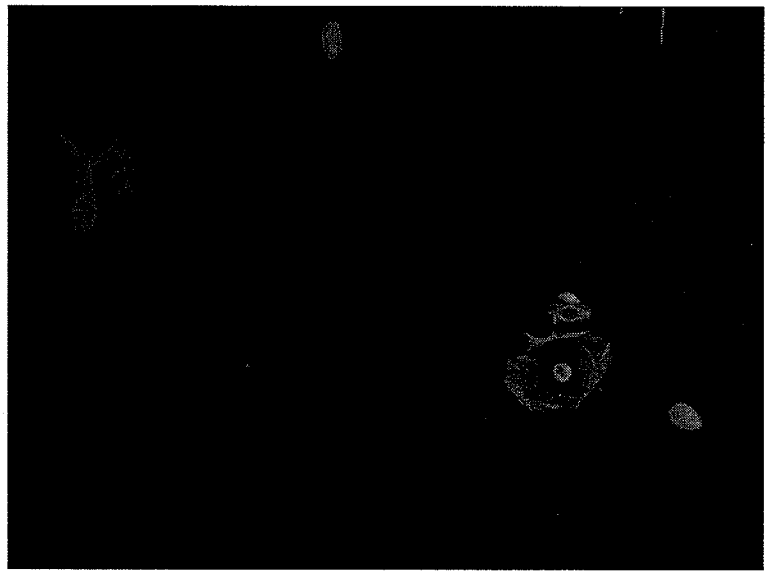
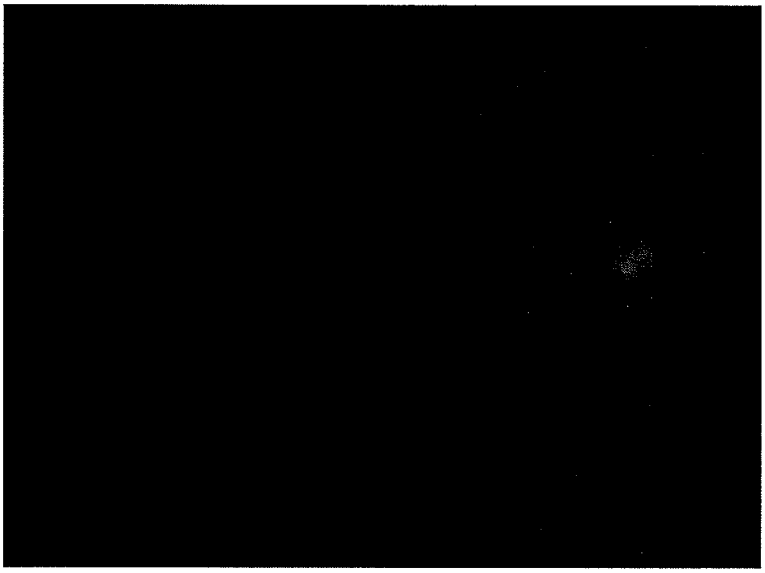


Figure 3B. Stained with antibodies against Amylase (green) and with DAPI



Appendix A

AN Dr. Matzaweyel

INSTITUT FÜR IMMUNOLOGIE UND GENETIK
im Klinikum Kaiserslautern

LABORARZTPRAXIS
Dr. med. Bernhard Thiele
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PD Dr. Charli Kruse

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Name:	**Lübeck Zellkultur**
Geb.:	SSW:
Labornummer:	04-18112
Eingangsdatum:	23.04.04
Befunddatum:	04.05.04
Pat.Nummer:	
Seite:	1/1

ZYTOGENETISCHER BEFUNDBERICHT

Material: Zellkultur

Chromosomenanalyse

Metaphasen - numerisch/grobstrukturell:	21 (1x44,2x45,18x46 Chromosomen)
Metaphasen - feinstrukturell:	10
Bänderungstechnik:	GTG-Banden
Bandenauflösung (ca.):	450
Karyotyp(en):	46,XX,22s+ [cp10]

Beurteilung:

Numerisch und strukturell unauffälliger weiblicher Karyotyp, bei der Satellitenverlängerung an einem Chromosom 22 handelt es sich um eine Normvariante ohne pathologische Relevanz.

Dr. med. B. Thiele

Endbefund